FIELD OF THE INVENTION

The instant invention relates to novel humanin-like polypeptides and polynucleotides encoding the same, and anti-humanin-like antibodies and their variants. The invention also describes the therapeutic activity of humanin in the treatment of neurodegenerative diseases.

BACKGROUND OF THE INVENTION

Neurodegenerative diseases present a rapidly increasing financial burden as western societies experience a rapid increase in the size of their aging population. The most common neurodegenerative disease is Alzheimer's disease (AD), which involves the parts of the brain that control thought, memory, and language. Symptoms usually start with forgetfulness and progress to difficulties in speaking and understanding. AD is a slow disease, starting with mild memory problems and ending with severe brain damage. The course the disease takes and how fast changes occur vary from person to person. On average, AD patients live from 8 to 10 years after they are diagnosed, though the disease can last for as long as 20 years. Abnormal clumps (amyloid plaques) and tangled bundles of fibers (neurofibrillary tangles) are seen in the brains of Alzheimer's patients, along with reduced levels of neurotransmitters in cortical cells. Despite current therapeutics, all Alzheimer's patients will eventually require total care. If the individual has no other serious illness, the loss of brain function itself will cause death.

No currently available treatment can stop AD. Tacrine (Cognex), donepezil (Aricept), rivastigmine (Exelon), or galantamine (Reminyl) may help prevent some symptoms from becoming worse in the early stages for a limited time. Only about half of the people taking these medications show modest and temporary improvement in memory and thinking skills. Also, some medicines may help control behavioral symptoms of AD such as sleeplessness, agitation, wandering, anxiety, and depression. Treating these symptoms often makes patients more comfortable and makes their care easier for caregivers. NSAIDs, vitamin E, and estrogen may also be of limited use to slow disease progress, but recent studies have not proved encouraging.

Parkinson's disease is a neurodegenerative disease that is clinically characterized by decrease in spontaneous movements, gait difficulty, postural instability, rigidity and tremor. Parkinson's disease affects one of every 100 persons over the age of 60. Parkinson's disease occurs when dopaminergic neurons of the substantial nigra begin to malfunction and eventually die. Dopamine is a neurotransmitter that is involved in control of movement initiation and coordination. The degeneration of dopaminergic neurons creates a shortage of the neurotransmitter dopamine, causing the movement impairments that characterize the disease. While several treatment options are currently available, none prevent the progressive changes of the brain typical of Parkinson's disease.

Hashimoto et al. (Proc. Nat. Acad. Sci. 98: 6336-6341, 2001) noted that important clues in the development of therapy for Alzheimer's disease (AD; OMIM104300) come from the study of molecules that suppress familial AD gene-induced death in neuronal cells in culture. Using the death-trap screening method devised by Vito et al. (Science 271: 521-524, 1996), they identified a cDNA, which they called humanin (HN), encoding a deduced 24-amino acid secretory polypeptide that suppresses neuronal cell death induced by mutations in 3 familial AD genes: amyloid precursor protein (APP; OMIM104760), presenilin-1 (PS1; OMIM104311), and presenilin-2 (PS2; OMIM600759). The peptide also abolished death caused by A-beta amyloid, but had no effect on death by Q79 or superoxide dismutase-1 mutants. Transfected HN cDNA was transcribed to the corresponding polypeptide and then was secreted into the cultured medium. The rescue action clearly depended on the primary structure of HN. Northern blot analysis detected expression of major 1.6- and minor 3.0- and 1.0-kb transcripts at high levels in heart, skeletal muscles, kidney, and liver, at lower but significant levels in brain and the gastrointestinal tract, and at barely detectable levels in the immune system.

Tajima et al. (*Neurosci. Lett.* 324: 227-231, 2002) raised an anti-HN antibody and found that long cDNAs containing the ORF of HN (HN-ORF) produced the HN peptide in mammalian cells, dependent on the presence of the full-length HN-ORF. Immunoblot analysis detected a 3-kD protein with HN immunoreactivity in mouse testes and colon. The findings suggested that the HN peptide could be produced in vivo. HN immunoreactivity was detected in neurons and glia of AD brains, whereas few immunopositive neuronal cells were detected in normal brains.

Cerebral amyloid beta-protein angiopathy (CAA) is a pathological feature of AD and related disorders. Jung and Van Nostrand (J Neurochem. 2003; 84(2):266-72) used human cerebrovascular smooth muscle cells as a model of CAA. They demonstrated that humanin could protect human cerebrovascular smooth muscle cells from A-beta-induced toxicity.

The novel humanin-like polypeptides (CuraGen accno: CG202524) of the present invention differ from the humanin polypeptides of the prior art (GenBank Acc.No. AY029066; PCT Publication WO200121787; European Publication EP01221480 A1) as follows. CG202524-02 has a Leucine to Serine amino acid substitution at position 12 (L12S). CG202524-03 has 2 amino acid substitutions: L12S, and an Alanine to Threonine substitution at position 24 (A24T). CG202524-04 has 3 amino acid substitutions: L12S, an Arginine to Leucine amino acid substitution at position 23 (R23L), and an Alanine to Leucine substitution at position 24 (A24L). Furthermore, CG202524-04 has 4 additional amino acids (SSVF) at positions 25 to 28, as compared to humanin (AAK50430). Thus, 3 novel CG202524 polypeptides have a Serine at position 12 (S12). A systematic site-directed mutagenesis analysis of Humanin has been disclosed in EP01221480, showing that replacement of Leucine at position 12 (L12) with Ala abolished the protective function of Humanin. The

inventor concluded that the L12 residue is essential for protection against cell death. However, the current invention is unique in that CG202524-02, -04 and –03 (each having S12) protected neurons from cell death under various conditions. Furthermore, another humanin-like polypeptide (CG202524-08) has 5 amino acid substitutions: S12L and T24A, S14T, E15A and I16T as compared to the known humanin (GenBank AY029066). The instant application further describes the use of the novel humanin-like compositions (polypeptide, polynucleotide and antibodies) in neurodegenerative diseases including, but not limited to, Parkinson's disease and Alzheimer's disease.

SUMMARY OF THE INVENTION

The present invention discloses a novel protein and nucleic acids, herein generally referred to by the identifier CG202524, bearing sequence similarity to Humanin, fragments thereof, and antibodies that bind immunospecifically to a protein of the invention.

One of the aspects of the present invention is to describe the therapeutic use of the polypeptide in preventing or inhibiting death of neuronal or cerebrovascular smooth muscle cells. More specifically, the novel compositions detailed in the present invention are useful in the prevention of cell death associated with neurodegenerative diseases.

Another aspect of the present invention is to describe a method of treating or preventing patients suffering from, or believed to be at risk of, a neurodegenerative disease or a condition of the central nervous system associated with neuronal death. The disease or disorder may include but is not limited to Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, spinocerebellar ataxia, amyotrophic lateral sclerosis, muscular dystrophy, peripheral neuropathy, traumatic head or spinal cord injury, and stroke.

Another aspect of the invention is to provide a method of preventing, reducing, or slowing the progression of, symptoms of neurodegenerative disease, or symptoms of a condition of the central nervous system associated with neuronal death. These symptoms may include but are not limited to cognitive impairment, learning deficit, memory deficit, memory loss, motor function impairment and mood disorder.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts a time course of cell death in rat PC2 cell cultures in response to serum withdrawal. Cell death was assessed by a fluorescent assay of lactate dehydrogenase concentration in the culture supernatants. The results show that serum starvation causes a time dependent increase in PC-12 cell death.

Figure 2a depicts the effect of different concentrations of HN-01 (CG202524-01) on cell death in rat PC2 cell cultures in response to serum withdrawal. HN-01 (CG202524-01) rescues PC12 cells from serum withdrawal-induced cell death in a dose-dependent manner.

Figure 2b depicts the effect of different concentrations of HN-06 (CG202524-02) on cell death in rat PC2 cell cultures in response to serum withdrawal. HN-06 (CG202524-02) rescues PC12 cells from serum withdrawal-induced cell death in a dose-dependent manner.

Figure 2c depicts the effect of different concentrations of HN-03 (CG202524-03) on cell death in rat PC2 cell cultures in response to serum withdrawal. HN-03 (CG202524-03) rescues PC12 cells from serum withdrawal-induced cell death in a dose-dependent manner.

Figure 2d depicts the effect of different concentrations of HN-07 (CG202524-04) on cell death in rat PC2 cell cultures in response to serum withdrawal. HN-07 (CG202524-04) rescues PC12 cells from serum withdrawal-induced cell death in a dose-dependent manner.

Figure 3 depicts the effects of novel humanin-like polypeptides on PC12 survival after serum withdrawal. Relative fluorescence units were normalized to the maximal fluorescence units observed under no HN treatment. All humanin-like polypeptides tested showed similar efficiency in protecting PC12 cells from serum withdrawal-induced cell death.

Figure 4 depicts the effects of novel humanin-like polypeptides on Dopamine-induced cell death. Relative fluorescence units indicate LDH level in supernatant released from dead cells, and were normalized to the maximal fluorescence units observed under no HN treatment. All humanin-like polypeptides tested showed similar efficiency in protecting PC12 cells from dopamine-induced toxicity.

Figure 5 depicts the effects of novel humanin-like polypeptides on CREB-3-mediated cell death. Relative fluorescence units indicate LDH level in supernatant released from dead cells. All humanin-like polypeptides tested showed similar efficiency in protecting PC12 cells from CREB-3-mediated cell death.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based upon the discovery of novel humanin-like polypeptides and polynucleotides encoding them. The identifier CG202524 herein generally refers to the novel humanin-like polypeptides and polynucleotides encoding them.

The following Table 1 provides a summary of the CG202524 nucleic acids and their encoded polypeptides.

Table 1. Novel Humanin-like polypeptides and polynucleotides

Internal Identification	SEQ ID NO (nucleic acid)	SEQ ID NO (amino acid)	Homology
CG202524-07	1	2	Humanin - Homo sapiens
CG202524-01	3	4	Humanin - Homo sapiens
CG202524-02	5	6	Humanin - Homo sapiens
CG202524-03	7	8	Humanin - Homo sapiens
CG202524-04	9	10	Humanin - Homo sapiens
CG202524-05	11	12	Humanin - Homo sapiens
CG202524-06	13	14	Humanin - Homo sapiens

CG202524-08	15	16	Humanin - Homo sapiens

Sequence homology search strategies were used to identify several additional chromosomal gene loci coding for humanin-like polypeptides. Five additional loci encoding humanin-like polypeptides were found in human genomic sequences from chromosomes 3, 6, 11, 17 and 5. The sequences of Acc. No. CG202524-02, CG202524-03 and CG202524-04, CG202524-06, CG202524-07 and CG202524-08 were derived by in silico prediction of the sequence based on sequences available in the public human sequence databases. One or more genomic clones AC117444.6 on chromosome 3, AC021914.7 on chromosome 11, AC131055.9 on chromosome 17, and AC008434.5 on chromosome 5 were identified by TBLASTN using CuraGen Corporation's sequence file for members of Humanin Programs used for these

The novel humanin-like polypeptides of the present invention differ from the humanin polypeptides of the prior art (see GenBank Acc.No. AY029066; WO200121787; European Publication EP01221480 A1). The novel humanin-like gene loci on chromosome 3 and 11 each encode a polypeptide (CG202524-02), which has a Leucine to Serine amino acid substitution at position 12 (L12S). A locus on chromosome 17 encodes another humanin related polypeptide (CG202524-03), which has 2 amino acid substitutions: L12S, and an Alanine to Threonine substitution at position 24 (A24T). A locus on chromosome 5 encodes yet another humanin like polypeptide (CG202524-04) with 3 amino acid substitutions: L12S, an Arginine to Leucine amino acid substitution at position 23 (R23L), and an Alanine to Leucine substitution at position 24 (A24L). Furthermore, CG202524-04 has 4 additional amino acids (SSVF) at positions 25 to 28, as compared to humanin (AAK50430). Thus, each of the three 3 novel polypeptides of the invention have a Serine at position 12 (S12). A systematic site-directed mutagenesis analysis of Humanin has been disclosed in EP01221480, showing that replacing Leucine at position 12 (L12) with Alanine abolished the protective function of Humanin. The inventor concluded that the L12 residue is essential for protection against cell death by humanin. However, the current invention is unique in that CG202524-02, -04 and –03 (having a Serine residue at position 12) has shown protection of neurons from cell death under various conditions. This is supported by the data presented in the instant application.

The observation that there are 4 genomic loci, which encode a Serine in place of Leucine at position 12, indicates a potential significance of this amino acid. For example, it has been shown that substitution of Serine at position 14 with Glycine potentiated the neuroprotective activity of Humanin thousand fold, whereas the substitution to Ala nullified the protective activity (see Proc. Nat. Acad. Sci. 98: 6336-6341, 2001). It is conceivable that substitution of L12 with Serine (found in 4 different genetic loci, and in 3 novel Humanin-like polypeptides described here) potentiates the neuroprotective activity of humanin. It is also possible that the replacement of Ala 24 with Thr in the CG202524-02 amino acid sequence,

and Arg23, Ala24 with Leu-Leu-Ser-Ser-Val-Phe in the CG202524-04 amino acid sequence has beneficial potentiating neuroprotective activities.

An additional locus was found in human genomic sequence from chromosome 6, which encodes a humanin- like polypeptide (CG202524-08) having 5 amino acid substitutions, S12L and T24A, S14T, E15A and I16T, as compared to the known humanin GenBank AY029066. CG202524-08 retains the Ser in the 12th position as compared to Leucine in the known humanin.

The following Table 2A provides the specific DNA and amino acid sequences for the nucleic acids and polypeptides of Table 1.

Table 2A. CG202524 Sequence Analyses

CG202524-07 DNA Sequence	SEQ ID NO: 1	90 bp
	ORF Start: ATG at 4	ORF Stop: TAG at 88

ACC**ATG**GCTCCACGAGGGTTCAGCTGTCTCTTACTTTCAACCAGTGAAATTGACCTGCCCGTGAAGAG
ACTTTTAAGTTCAGTTTTT**TAG**

CG202524-07 Protein Sequence	SEQ ID NO: 2	28 aa	MW at 3080.6kD

MAPRGFSCLLLSTSEIDLPVKRLLSSVF

CG202524-01 DNA Sequence	SEQ ID NO: 3	1567 bp
	ORF Start: ATG at 951	ORF Stop: TAA at 1023

CCCAAACCCACTCCACCTTACTACCAGACAACCTTAGCCAAACCATTTACCCAAATAAAGTATAGGCG
ATAGAAATTGAAACCTGGCGCAATAGATATAGTACCGCAAGGGAAAGATGAAAAATTATAACCAAGCA
TAATATAGCAAGGACTAACCCCTATACCTTCTGCATAATGAATTAACTAGAAATAACTTTGCAAGGAG
AGCCAAAGCTAAGACCCCCGAAACCAGACGAGCTACCTAAGAACAGCTAAAAGAGCACACCCGTCTAT
GTAGCAAAATAGTGGGAAGATTTATAGGTAGAGGCGACAAACCTACCGAGCCTGGTGATAGCTGGTTG
TCCAAGATAGAATCTTAGTTCAACTTTAAATTTGCCCACAGAACCCTCTAAATCCCCTTGTAAATTTA
ACTGTTAGTCCAAAGAGGAACAGCTCTTTGGACACTAGGAAAAAACCTTGTAGAGAGAGTAAAAAATT
TAACACCCATAGTAGGCCTAAAAGCAGCCACCAATTAAGAAAGCGTTCAAGCTCAACACCCACTACCT
AAAAAATCCCAAACATATAACTGAACTCCTCACACCCAATTGGACCAATCTATCACCCTATAGAAGAA
CTAATGTTAGTATAAGTAACATGAAAACATTCTCCTCCGCATAAGCCTGCGTCAGATTAAAACACTGA
ACTGACAATTAACAGCCCAATATCTACAATTAACCAACAAGTCATTATTACCCTCACTGTCAACCCAA
CACAGGCATGCTCATAAGGAAAGGTTAAAAAAAGTAAAAGGAACTCGGCAAATCTTACCCCGCCTGTT
TACCAAAAACATCACCTCTAGCATCACCAGTATTAGAGGCACCGCCTGCCCAGTGACACATGTTTAAC
GGCCGCGGTACCCTAACCGTGCAAAGGTAGCATAATCACTTGTTCCTTAATTAGGGACCTGTATGA**AT**
GGCTCCACGAGGGTTCAGCTGTCTCTTACTTTTAACCAGTGAAATTGACCTGCCCGTGAAGAGGCGGG
CA**TAA**CACAGCAAGACGAGAAGACCCTATGGAGCTTTAATTTATTAATGCAAACAGTACCTAACAAAC
CCACAGGTCCTAAACTACCAAACCTGCATTAAAAATTTCGGTTGGGGCGACCTCGGAGCAGAACCCAA
CCTCCGAGCAGTACATGCTAAGACTTCACCAGTCAAAGCGAACTACTATACTCAATTGATCCAATAAC
TTGACCAACGGAACAAGTTACCCTAGGGATAACAGCGCAATCCTATTCTAGAGTCCATATCAACAATA
GGGTTTACGACCTCGATGTTGGATCAGGACATCCCAATGGTGCAGCCGCTATTAAAGGTTCGTTTGTT
CAACGATTAAAGTCCTACGTGATCTGAGTTCAGACCGGAGTAATCCAGGTCGGTTTCTATCTACTTCA
AATTCCTCCCTGTACGAAAGGACAAGAGAAATAAGGCCTACTTCACAAAGCGCCTTCCCCCGTAAATG
ATATCATCTCAACTTAGTATTATACCCACACCCACCCAAGAACAGGGTTTGTTAAGAAAAAAAAAAAA
AAA

CG202524-01 Protein Sequence	SEQ ID NO: 4	24 aa	MW at 2687.2kD

MAPRGFSCLLLLTSEIDLPVKRRA

CG202524-02 DNA Sequence	SEQ ID NO: 5	75 bp
	ORF Start: ATG at 1	ORF Stop: TAA at 73

ATGGCTCCACGAGGGTTCAGCTGTCTCTTACTTTCAACCAGTGAAATTGACCTACCCGTGAAGAGGCG
GGCA**TAA**

CG202524-02 Protein Sequence	SEQ ID NO: 6	24 aa	MW at 2661.1kD
MAPRGFSCLLLSTSEIDLPVKRRA			

CG202524-03 DNA Sequence	SEQ ID NO: 7	75 bp
	ORF Start: ATG at 1	ORF Stop: TAA at 73
ATGGCTCCACGAGGGTTCAGCTGTCTCTTACTTTCAACCAGTGAAATTGACCTGCCCGTGAAGAGGCG GACA**TAA**		

CG202524-03 Protein Sequence	SEQ ID NO: 8	24 aa	MW at 2691.1kD
MAPRGFSCLLLSTSEIDLPVKRRT			

CG202524-04 DNA Sequence	SEQ ID NO: 9	87 bp
	ORF Start: ATG at 1	ORF Stop: TAA at 85
ATGGCTCCACGAGGGTTCAGCTGTCTCTTACTTTCAACCAGTGAAATTGACCTGCCCGTGAAGAGACT TTTAAGTTCAGTTTTT**TAA**		

CG202524-04 Protein Sequence	SEQ ID NO: 10	28 aa	MW at 3080.6kD
MAPRGFSCLLLSTSEIDLPVKRLLSSVF			

CG202524-05 DNA Sequence	SEQ ID NO: 11	78 bp
	ORF Start: ATG at 4	ORF Stop: TAG at 76
ACC**ATG**GCTCCACGAGGGTTCAGCTGTCTCTTACTTTTAACCAGTGAAATTGACCTGCCCGTGAAGAG GCGGGCA**TAG**		

CG202524-05 Protein Sequence	SEQ ID NO: 12	24 aa	MW at 2687.2kD
MAPRGFSCLLLLTSEIDLPVKRRA			

CG202524-06 DNA Sequence	SEQ ID NO: 13	79 bp
	ORF Start: ATG at 4	ORF Stop: TAG at 76
ACC**ATG**GCTCCACGAGGGTTCAGCTGTCTCTTACTTTCAACCAGTGAAATTGACCTACCCGTGAAGAG GCGGGCA**TAG**C		

CG202524-06 Protein Sequence	SEQ ID NO: 14	24 aa	MW at 2661.1kD
MAPRGFSCLLLSTSEIDLPVKRRA			

CG202524-08 DNA Sequence	SEQ ID NO: 15	75 bp
	ORF Start: ATG at 1	ORF Stop: TAA at 73
ATGGCTCGACGAGGTTTCAGCTGTCTCTTACTTTCAACCACTGCAACTGACCTGCCCGTGAAGAGGCG GACA**TAA**		

CG202524-08 Protein Sequence	SEQ ID NO: 16	24 aa	MW at 2694.2kD
MARRGFSCLLLSTTATDLPVKRRT			

A ClustalW comparison of the above protein sequences yields the following sequence alignment shown in Table 2B.

Table 2B. Comparison of the CG202524 protein sequences.

```
CG202524-01  1  MAPRGFSCLLLLTSEIDLPVKRRA----  24
CG202524-05  1  MAPRGFSCLLLLTSEIDLPVKRRA----  24
CG202524-02  1  MAPRGFSCLLLSTSEIDLPVKRRA----  24
CG202524-06  1  MAPRGFSCLLLSTSEIDLPVKRRA----  24
CG202524-04  1  MAPRGFSCLLLSTSEIDLPVKRLLSSVF  28
CG202524-07  1  MAPRGFSCLLLSTSEIDLPVKRLLSSVF  28
CG202524-03  1  MAPRGFSCLLLSTSEIDLPVKRRT----  24
CG202524-08  1  MARRGFSCLLLSTTATDLPVKRRT----  24
```

According to the present invention:

- CG202524-02 and –06 are novel humanin-like polypeptides and polynucleotides encoding them. CG202524-02 and –06 have the same protein sequences.
- CG202524-03 is a novel humanin-like polypeptide and the polynucleotide encoding it.
- CG202524-04 and –07 are novel humanin-like polypeptides and polynucleotides encoding them. CG202524-04 and –07 have the same protein sequences.
- CG202524-08 is a novel humanin-like polypeptide and the polynucleotide encoding it.
- CG202524-01 and –05 are the known humanin (GenBank: AY029066).

A search of the CG202524-07 protein according to the present invention against the Geneseq database, a proprietary database that contains sequences published in patents and patent publication, yielded several homologous proteins shown in Table 2C.

Table 2C. Geneseq Results for CG202524-07

Geneseq Identifier	Protein/Organism/Length [Patent #, Date]	CG202524-07 Residues/ Match Residues	Identities/ Similarities for the Matched Region	Expect Value
AAO30314	Human humanin (cytosolic form) peptide - Homo sapiens, 24 aa. [WO2003046205-A2, 05-JUN-2003]	1..22 1..22	21/22 (95%) 21/22 (95%)	1e-04
AAO30161	Human humanin protein, HN1 - Homo sapiens, 24 aa. [WO2003045988-A2, 05-JUN-2003]	1..22 1..22	21/22 (95%) 21/22 (95%)	1e-04
AAU69614	Cell death protective sequence CNI-00725, protein #4 - Homo sapiens, 24 aa. [WO200176532-A2, 18-OCT-2001]	1..22 1..22	21/22 (95%) 21/22 (95%)	1e-04

ABB44628	Human protective sequence CNI-00734 peptide #5 - Homo sapiens, 24 aa. [WO200176457-A2, 18-OCT-2001]	1..22 1..22	21/22 (95%) 21/22 (95%)	1e-04
AAU73274	Human protective DNA sequence CNI-00736 open reading frame #5 - Homo sapiens, 24 aa. [WO200181361-A1, 01-NOV-2001]	1..22 1..22	21/22 (95%) 21/22 (95%)	1e-04

In a BLAST search of public sequence databases, the CG202524-07 protein according to the present invention was found to have homology to the proteins shown in the BLASTP data in Table 2D.

Table 2D. Public BLASTP Results for CG202524-07

Protein Accession Number	Protein/Organism/Length	CG202524-07 Residues/ Match Residues	Identities/ Similarities for the Matched Portion	Expect Value
Q8IVG9	Humanin - Homo sapiens (Human), 24 aa.	1..22 1..22	21/22 (95%) 21/22 (95%)	2e-04

As would be understood by those skilled in the art, a nucleic acid or amino acid sequence homologous to the CG202524 sequences disclosed herein could be used in the method of the invention. "Homologous" refers to sequences characterized by a homology at the nucleotide level or amino acid level and include sequences coding for isoforms such as those expressed in different tissues of the same origin encoded for example by alternative splicing of RNA. Alternatively, isoforms can be encoded by different genes. In the invention, homologous sequences include CG202524 polypeptides and the nucleotide sequences encoding them, of species other than humans, including, but not limited to vertebrates, and thus can include, *e.g.*, frog, mouse, rat, rabbit, dog, cat, cow, horse, and other organisms. Homologous sequences also include naturally occurring allelic variations and mutations of the sequences set forth herein. Homologous sequences include those sequences having conservative amino acid substitutions and the nucleotides encoding them.

A polypeptide having a biologically active portion of a CG202524 polypeptide has an activity of CG202524 as measured in a particular biological assay (such as those described herein). Such biologically active polypeptides can be used in the method of the invention. A nucleic acid fragment encoding a biologically-active polypeptide can be prepared by isolating

a portion of the nucleotide that encodes a polypeptide having a CG202524 biological activity, expressing the encoded portion of CG202524 (*e.g.*, by recombinant expression *in vitro*) and assessing the activity of the encoded portion of CG202524.

In addition to naturally-occurring allelic variants of CG202524 sequences, the skilled artisan will further appreciate that changes may be introduced into the nucleotide sequences that may lead to changes in the amino acid sequences of the encoded CG202524 protein, without altering the functional ability of that protein. Such proteins also have utility in the method of the invention. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in the sequence. A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of the protein without altering its biological activity, whereas an "essential" amino acid residue is required for such biological activity. Amino acids for which conservative substitutions can be made are well-known within the art. Polypeptides that contain changes in amino acid residues of CG202524 polypeptides that are not essential for activity may also be used in the method of the invention.

Chimeric or fusion proteins including CG202524 polypeptides may be used in the method of the invention. As used herein, a "chimeric protein" or "fusion protein" comprises a CG202524 polypeptide operatively-linked to a non-CG202524 polypeptide. Within such a fusion protein the CG202524 polypeptide can correspond to all or a portion of a CG202524 protein. In one embodiment, a CG202524 fusion protein comprises at least one biologically-active portion of a CG202524 protein. Within the fusion protein, the CG202524 polypeptide and the non-CG202524 polypeptide are "operatively-linked", that is they are fused in-frame with one another. The non-CG202524 polypeptide can be fused to the N-terminus or C-terminus of the CG202524 polypeptide. For example, the fusion protein may be a CG202524 protein containing a heterologous signal sequence at its N-terminus. In certain host cells (*e.g.*, mammalian host cells), expression and/or secretion of CG202524 can be increased through use of a heterologous signal sequence. In yet another example, the fusion protein is a CG202524-immunoglobulin fusion protein in which the CG202524 sequences are fused to sequences derived from a member of the immunoglobulin protein family. The CG202524-immunoglobulin fusion proteins can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an immunological response according to the present invention.

A CG202524 chimeric or fusion protein for use in the method of the invention may be chemically modified for the purpose of improving bioavailability, and increasing efficacy, solubility and stability. For example, the protein may be covalently or non-covalently linked to polyethylene glycol (PEG), or by other well-known methods in the art.

A CG202524 chimeric or fusion protein for use in the method of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences may be ligated together in-frame in accordance with

conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. Furthermore, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence [*see, e.g.,* Ausubel, *et al.* (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, (1992)]. Moreover, many expression vectors are commercially available that already encode a fusion moiety (*e.g.*, a GST polypeptide). A CG202524-encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the CG202524 protein. Furthermore, the chimeric or fusion protein may be produced synthetically. The fusion protein can be a CG202524 protein fused to a His tag or epitope tag (e.g. V5) to aid in the purification and detection of the recombinant CG202524 protein.

In one use, the present invention provides CG202524 proteins, analogs and homologs that can be incorporated into pharmaceutical compositions suitable for administration for patient use. Such compositions comprise the CG202524 protein, either alone or together with one or more conventional pharmaceutically acceptable carriers, such as solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound which is the CG202524 protein as described herein, use thereof in the compositions is contemplated. Supplementary active compounds may also be incorporated into the compositions.

In another embodiment, the method uses a pharmaceutical composition formulated to be compatible with its intended route of administration. Examples of routes of administration include but not limited to parenteral i.e intravenous, intravenous, intracranial and intracerebral, transmucosal or transdermal administration. Solutions or suspensions used for parenteral, intradermal, or subcutaneous application may include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and agents for the adjustment of tonicity such as sodium chloride or dextrose. The pH may be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation may be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

By intranasal administration, it is meant that the compounds are delivered in the form of an aerosol spray from pressured container or dispenser, which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer. Systemic administration may also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration may be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

By pharmaceutical composition, it is meant that a composition that is utilized for the administration into a mammal, or more specifically human. Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, or phosphate buffered saline (PBS). The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity may be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms may be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it is be preferable to include isotonic agents, for example, sugars, polyalcohols such as manitol, sorbitol, and sodium chloride in the composition

By carriers, as used herein it is meant that the composition is prepared with carriers that protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers may be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) may also be used as pharmaceutically acceptable carriers. These may be prepared according to methods well-known to those skilled in the art.

The data included herein support the use of the nucleic acids, polypeptides and antibodies in the treatment of neurodegenerative diseases as described above. Briefly the present invention provides a novel Humanin-like polypeptides and polynucleotides encoding them that prevent cell death associated with neurodegenerative diseases, and use of the same. Furthermore, the present invention describes compositions comprising a therapeutic

polypeptide, CG202524, useful in the prevention of cell death. More specifically, the novel compositions detailed in the present invention are useful in the prevention of cell death associated with neurodegenerative diseases.

Additionally, the present invention provides a method of treating subjects suffering from, or believed to be at risk of, a neurodegenerative disease or a condition of the central nervous system associated with neuronal death. The disease or condition may include but is not limited to Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, spinocerebellar ataxia, amyotrophic lateral sclerosis, muscular dystrophy, peripheral neuropathy, traumatic head or spinal cord injury, and stroke. Another aspect of the invention is a method of reducing, or slowing the progression of, symptoms of neurodegenerative disease. These symptoms may include but are not limited to cognitive impairment, learning deficit, memory deficit, memory loss, motor function impairment, tremor and mood disorder. Further, a method of administering a pharmaceutical composition comprising humanin polypeptides to reduce neuronal death resulting from ischemic insult, stroke, or prion-induced disease and to treat or prevent cerebral amyloid beta-protein ((Abeta) angiopathy in Alzheimer's disease (AD) and related disorders are also described herein.

EXAMPLES

Example 1

Serum withdrawal-induced toxicity.

The therapeutic potential of the humanin-like polypeptides of the present invention for neurodegenerative diseases was evaluated in vitro. Serum is critical for in vitro growth and survival of several populations of primary neurons and neuronal cell lines, such as PC12 cells. It has been used broadly as a primitive neuronal cell death model. PC12 cell is a rat pheochromocytoma cell line that has been widely used as a cell system for neuronal signaling and differentiation due to its ability to alter its phenotype to a sympathetic neuron-like cell in response to nerve growth factor or fibroblast growth factor. Therefore, determining whether the CG202524 variants rescued PC12 from cell death induced by serum withdrawal provided insight on the role of these proteins in neuronal protection.

A rapid, fluorescence-based LDH assays was used to measure the release of lactate dehydrogenase from cells with a damaged membrane. Briefly, PC12 cells were plated in 96 wells plate in complete serum. After 24 hours, cells were washed with serum-free media twice and were cultured in serum-free media together with a CG202524 variants at various concentrations. Cell supernatants were collected and subjected to LDH assays (CytoTox-ONE™, Promega, WI) at 24 or 48 hrs after treatment. The reactions were terminated by adding a stop buffer and the signal measured using a fluorescence reader. Higher read of LDH signal indicated more cell death occurring during the incubation time.

Results. Figure 1 shows the cell death response pattern of PC-12 upon serum starvation. The data presented indicates that serum starvation results in a time dependent increase in the PC-12 cell death. However, Humanin HN-01 (CG202524-01, known form) protein rescued the PC-12 cells from serum starvation induced cell death (Figure 2a). Similar results were obtained with the novel humanin-like polypeptides HN-06 (CG202524-02 also known as -06), HN-03 (CG202524-03), HN-07 (CG202524-04 also known as -07) as seen in Figures 2b, 2c and 2d respectively. Also the effect of survival of PC-12 was comparable in the presence of all the novel variants identified (Figure 3). These results collectively show that the novel humanin-like polypeptides according to the present invention have the capability to protect neurons from death and thus can be effective as therapeutics for neurodegenerative diseases.

Example 2

Dopamine-induced toxicity

Dopamine induces cell toxicity in primary neurons and several neuronal cell lines, such as PC12 cells. It has been implicated that Dopamine toxicity is partially responsible for neuronal cell death in Alzheimer's, Parkinson's disease and several other neurodegenerative diseases. Therefore, determining whether a Humanin-like variants described herein, rescues PC12 cell death induced by Dopamine will provide important information about the role of the proteins in neuron protection.

A rapid, fluorescence-based LDH assay was used to measure the release of lactate dehydrognease from PC-12 cells with a damaged membrane as described in Example 1. Again, all the humanin-like polypeptides tested, HN-01, HN-06, HN-03, HN-07, showed comparable efficiency in protecting the PC-12 cells from dopamine-induced toxicity (Figure 4). These results further emphasize the role of the novel CG202524 polypeptides in neuronal protection and the therapeutic potential of the peptides in neurodegenerative diseases.

Example 3

CREB-3 mediated toxicity

The goal of this experiment was to assess the survival of PC-12 cells in the presence of Humanin-like polypeptides (CG202524-01, CG202524-02, CG202524-04). Cells were transiently cotransfected with a dominant interfering CREB-3 plasmid and plasmids encoding different isoforms of HNs. LDH level in supernatant released from dead cells was determined by LDH assay as described in Examples 1 and 2. The control without humanin polypeptide showed elevated levels of cell death. In the presence the CG202524 polypeptide, cell death was decreased suggesting the protective role of the humanin-like polypeptides of the present invention in CREB-3 mediated cytotoxicity (Figure 5).

Example 4

Routes of Administration

To determine the most appropriate method delivery to the brain, compositions comprising CG202524 polypeptides are administered to rodents intravenously (IV), intracranially or intracerebrally (IC), or intranasally (IN). Humanin concentration is measured in the cerebrospinal fluid (CSF) at various time intervals following administration. The humanin concentration in CSF is used as a model of blood-brain barrier (BBB) crossing and stability. Humanin concentration is measured by immunological or radiological methods well known in the art.

IC administration may be achieved by one of several methods known in the art, including, i.e. by injection of humanin polypeptide in a suitable pharmacological carrier, by sustained release of humanin polypeptide from a polymer implant, or by use of a mini-osmotic pump (see Br J Cancer 2000, 82(1):74-80). Long-term IC administration may also be achieved by transplanting recombinant humanin-secreting fibroblasts into the CNS (see Clin Neurosci.1995-96, 3(5):268-74).

It has been shown that intranasal administration allows therapeutic polypeptides to cross the blood-brain barrier. (see Nat Neurosci 2002, 5(6):514-6; and Eur J Pharm Sci 2000, 11(1):1-18). Intranasal administration may be achieved by delivering the therapeutic polypeptides in the form of an aerosol, in a pharmaceutically suitable carrier. The aerosol may be produced by a variety of devices known to those skilled in the art, including but not limited to inhaler devices, nebulizers and vaporizers.

CG202524 is detected in CSF by one of several methods including specific anti-humanin antibodies, epitope tagging, biotinylation, and radiolabelling, all of which are techniques readily recognized by one skilled in the art. Stability of humanin in blood may also be determined by adding humanin polypeptide to blood serum in vitro, and measurement of Humanin concentration at various time intervals.

Example 5

Use of humanin-like polypeptides for prevention of neurodegeneration in animal models

To assess the potential of humanin as a therapeutic for neurodegenerative diseases, humanin-like polypeptides of the invention are administered to appropriate in vivo disease models. Animal models of neurodegenerative diseases are well known to those skilled in the art and exemplified by Examples 5A and 5B below.

Example 5A

Use of humanin-like polypeptides for prevention of neurodegeneration in an animal model of Parkinson's disease

To assess therapeutic utility of humanin-like polypeptides of the present invention in the treatment of Parkinson's Disease (PD), the polypeptide is administered to a rodent model of the disease. In one such model, PD-like symptoms are induced by unilateral stereotaxic injection of 6-hydroxy-dopamine into specific regions of the brain, such as the substantia nigra or the striatum (see Brain Res. 1992, 595(2):316-26). Humanin polypeptide is administered at a dose of 0.1 μM, 1μM, 10 mM or 100mM via one or more of the routes detailed in Example 4. Therapeutic outcome is measured by immunohistochemical measurement of tyrosine hydroxylase. Therapeutic outcome is further assessed by measurements of animal behavior, such as T-maze spontaneous alternation.

Example 5B

Use of humanin-like polypeptides for prevention of neurodegeneration in an animal model of Alzheimer's disease

To assess therapeutic utility of CG202524 polypeptides in the treatment of Alzheimer's disease (AD), the polypeptides are administered to a rodent model of the disease. In one such model, doubly transgenic mice overexpressing mutant APP and PS1 transgenes develop AD-like phenotypes including amyloid deposits and behavioral impairment as early as 10 weeks of age (see Neurobiol Dis. 1999, 6(4):231-44). CG202524 polypeptide is administered at a dose of 0.1 μM, 1μM, 10 mM or 100mM via one or more of the routes detailed in Example 4. Therapeutic outcome is assessed by measurements of amyloid plaques, and behavioral studies such as the Y - maze. Furthermore, markers of oxidative stress, including inflammation and neurodegeneration, are measured by methods well known to one skilled in the art.

Thus we have illustrated and described the preferred embodiment of our invention, it is to be understood that this invention is capable of variation and modification, and we therefore do not wish to be limited to the précis terms set forth, but desire to avail ourselves of such changes and alterations which may be made for adapting the invention t various usages and conditions. Such alterations and changes may include, for different compositions for the administration of the polypeptides according to the present invention to a mammal; different amounts of the polypeptide; different times and means of administration; different materials contained in the administration dose including, for example combinations of different peptides, or combinations of peptides with different biologically active compounds. Such changes and alterations also are intended to include modifications in the amino acid sequence of the specific polypeptides described herein in which such changes alter the sequence in a manner as not to change the functionality of the polypeptide, but as to change solubility of the peptide in the composition to be administered to the mammal, absorption of the peptide by the body, protection of the polypeptide for either shelf life or within the body until such time as the biological action of the peptide is able to bring about the desired effect, and such similar modifications. Accordingly, such changes and alterations are properly intended to be within the full range of equivalents, and therefore within the purview of the following claims. Having thus described our invention and the manner and process of making and using it in such full, clear, concise and exact terms so as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same.